

# Effects of endodontic tri-antibiotic paste on bond strengths of dentin adhesives to coronal dentin

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**Objectives:** The aim of this study was to evaluate the effects of tri-antibiotic paste (TAP) on microtensile bond strengths (MTBS) of dental adhesives to dentin. **Materials and Methods:** Sixty extracted molars had their occlusal surfaces flattened to expose dentin. They were divided into two groups, i.e., control group with no dentin treatment and experimental group with dentin treatment with TAP. After 10 days, specimens were bonded using self-etch (Filtek P90 adhesive) or etch-and-rinse (Adper Single Bond Plus) adhesives and restored with composite resin. Teeth were sectioned into beams, and the specimens were subjected to MTBS test. Data were analyzed using two-way ANOVA and *post hoc* Tukey tests. **Results:** There was a statistically significant interaction between dentin treatment and adhesive on MTBS to coronal dentin ( $p = 0.003$ ). Despite a trend towards worse MTBS being noticed in the experimental groups, TAP application showed no significant effect on MTBS ( $p = 0.064$ ). **Conclusions:** The etch-and-rinse adhesive Adper Single Bond Plus presented higher mean bond strengths than the self-etch adhesive Filtek P90, irrespective of the group. The superior bond performance for Adper Single Bond when compared to Filtek P90 adhesive was confirmed by a fewer number of adhesive failures. The influence of TAP in bond strength is insignificant. (*Restor Dent Endod* 2015;40(2):136-142)

**Key words:** Dentin; Etch-and-rinse adhesive; Microtensile bond strength; Self-etch adhesive

## Introduction

Medicaments other than calcium hydroxide and chlorhexidine have been investigated in the treatment of endodontic infections.<sup>1</sup> Antibiotic treatment, which may be a combination of metronidazole, ciprofloxacin, and minocycline, has been recommended in more complex situations in order to properly remove different pathogens.<sup>2,3</sup> That treatment, however, may affect the adhesion of the restorative material used for sealing of the endodontic access.<sup>4,5</sup>

There has been an increasing tendency to restore endodontically treated teeth with composite resins, either as foundation for indirect restoration or as final restoration.<sup>6</sup> To achieve a successful restoration, adequate adhesion to the tooth structure is critical. In that regard, dentin is a hydrated biologic complex that can be modified in the presence of various chemical agents.<sup>7</sup> The effects of endodontic materials such as antibiotic pastes and irrigation solutions on adhesion have been described.<sup>4,5</sup>

Complex root canal infections, which may not be fully resolved with a single antibiotic, often are treated with the tri-antibiotic paste (TAP).<sup>2</sup> Sato *et al.* first

tested the regimen with further studies confirming the antimicrobial efficacy of the ciprofloxacin-metronidazole-minocycline paste against root canal pathogens.<sup>2,8</sup> TAP has a low pH of 2.8, and its ability to chelate calcium has been shown to be similar to that of ethylenediamine tetraacetic acid (EDTA) in the studies.<sup>5,9,10</sup>

Adhesive procedures, often used in the process of restoring endodontically treated teeth, are divided into three steps of etch, prime, and bond. While etch-and-rinse adhesive systems have the three steps separately applied and require application of an etchant prior to priming, self-etch adhesives combine etching with priming.<sup>11,12</sup> Etch-and-rinse adhesives often do not fully penetrate the demineralized dentin leaving denuded collagen fibrils vulnerable to degradation by endogenous matrix metalloproteinases (MMPs).<sup>8,13</sup> Altering the dentin surface prior to adhesive procedures may limit their penetration into dentin and/or further increase the amount of exposed collagen fibrils, compromising adhesion. Likewise, bonding with self-etch adhesives may be compromised by altering the dentin surface and consequently limiting chemical adhesion.

The present study evaluated the effects of TAP on microtensile bond strengths (MTBS) of two types of dental adhesives to dentin. We hypothesized that TAP negatively affects bond strengths of etch-and-rinse and self-etch adhesives to coronal dentin.

## Materials and Methods

### Sample preparation

Subsequent to approval by the University Research Ethics Board (protocol 6452/2011), 60 extracted human third molars free of caries or any visible defects were collected and stored in 0.2% thymol solution at room temperature for up to 60 days.<sup>14</sup> Prior to the experiment, teeth were transferred to distilled water for 24 hours for removal of thymol residues. Each tooth was decoronated below the

cemento-enamel junction perpendicular to the longitudinal axis using a slow-speed, water-cooled diamond disc (D&Z, Berlin, Germany).<sup>15</sup> The occlusal enamel of each tooth was removed perpendicular to the long axis of the teeth with a diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water irrigation to expose superficial coronal dentin. Prepared dentin surfaces were finished with 600-grit silicon carbide paper under water irrigation to create a uniform smear layer.<sup>16</sup>

### Tri-antibiotic paste

Metronidazole (250 mg, Pars Darou Co., Tehran, Iran), minocycline chlorhydrate (100 mg, Biopharma Co., Roma, Italy), and ciprofloxacin chlorhydrate (250 mg, Arya Darou, Tehran, Iran) were vortexed with purified glycerin (vehicle) and methylcellulose (thickening agent) for 5 minutes to obtain a paste-like consistency. The mixture was adjusted to obtain a concentration of 20 mg/mL of the paste.

Specimens were randomly divided into two groups ( $n = 30$ ). In the control group, specimens were covered with cotton pellets moistened with saline. In the experimental group, dentin surfaces were treated using TAP (direct application onto the dentin surfaces). Specimens were then covered with cellophane to protect against contaminants and kept in an incubator at 37°C at relative humidity. After 10 days, dentin surfaces in both groups were rinsed using distilled water for 5 seconds in preparation for bonding procedures.

### Bonding procedures

Occlusal surfaces of all teeth were thoroughly rinsed with water and dried with oil- and moisture-free compressed air. Experimental and control groups were further divided into four subgroups ( $n = 15$ ) according to the adhesive system to be used (Figure 1). Information on the composite resins and adhesive systems used are summarized in Table 1.

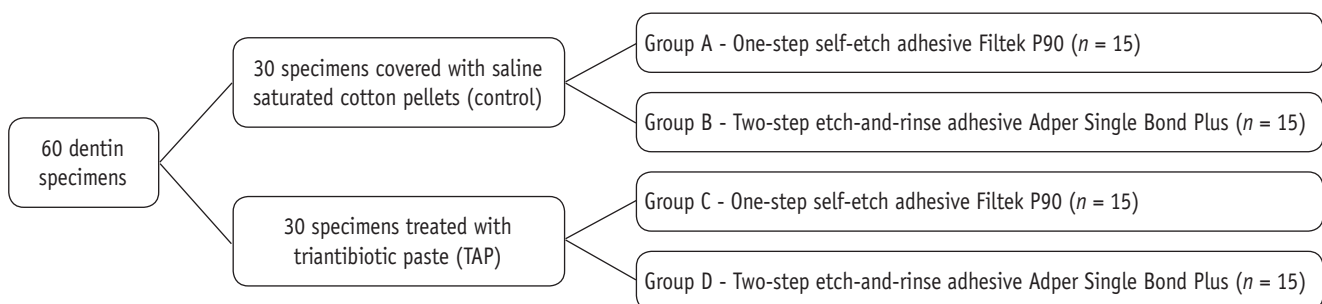


Figure 1. Flow chart of the experimental groups and procedures.

- Groups A and C: Filtek P90 (3M ESPE, St Paul, MN, USA) composite resin was used with its dedicated adhesive system (Filtek P90 adhesive). The self-etch primer was applied with gentle agitation using a microbrush for 20 seconds, gently air-thinned for 2 seconds, and polymerized for 10 seconds using a halogen light curing unit (Coltolux 2.5, Coltene AG, Feldwiesenstrasse Altstötten, Switzerland) with 600 mW/cm<sup>2</sup> output. The bonding resin was then applied and dispersed with a light stream of air, and light-cured for 20 seconds. P90 composite resin was built up in increments to a height of 5 mm to ensure sufficient bulk for the bond strength test. Each increment was light-cured for 40 seconds.
- Groups B and D: Filtek Z350 (3M ESPE) composite resin and Adper Single Bond Plus adhesive system (3M ESPE) were used. Dentin surfaces were acid-etched using 35% phosphoric acid gel (3M ESPE) for 15 seconds and rinsed for 30 seconds. Excess moisture was removed with absorbent paper (Aria Paper & Film Industries Co., Tehran, Iran) and the surface kept moist. A single coat of adhesive was immediately applied onto the prepared dentin surface with agitation for 30 seconds and then gently air-dried for 3 seconds prior to light curing for 20 seconds. Filtek Z350 composite resin build-ups were placed as described in groups A and C.

### Microtensile bond strength test

Specimens were stored in distilled water at 37°C for 24 hours and then sectioned using a diamond saw under water lubrication (Isomet), perpendicular to the

bonded interface. Two beams approximately 0.9 mm<sup>2</sup> in diameter from the central area of each specimen were collected ( $n = 30$  beams/group).<sup>17</sup> Beams were mounted on a universal testing machine (Instron, Norwood, MA, USA) using cyanoacrylate adhesive (Loctite, Loctite Ltd., Dublin, Ireland). The samples were stressed to failure at a crosshead speed of 0.5 mm/min (Figure 2). Results were expressed in MPa by dividing the resultant force in Newton by the cross-sectional area of the specimen. The bond strength data were statistically analyzed by two-way ANOVA and *post hoc* Tukey test using SPSS 15 ( $\alpha = 0.05$ ).

### Failure mode

Failure mode was determined with a stereomicroscope (Olympus, Tokyo, Japan) and classified as adhesive, adhesive failure between adhesive and dentin; cohesive, failure in composite resin or dentin; mixed, combination of adhesive and cohesive failures.<sup>18</sup>

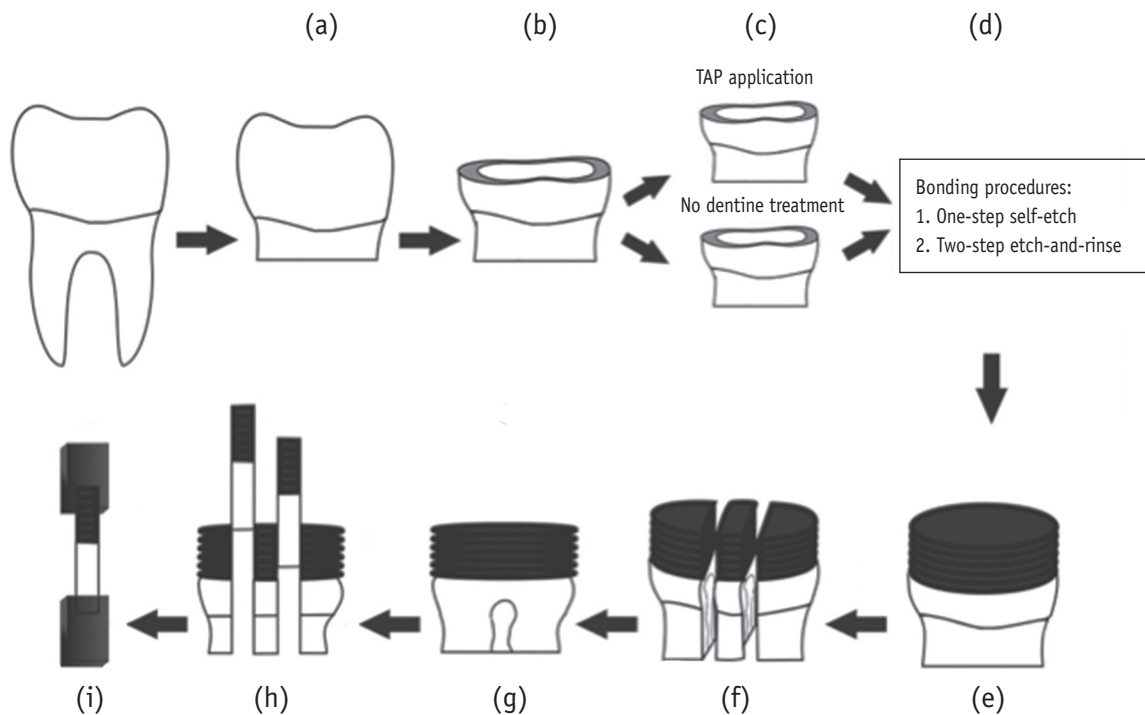
### Results

The mean MTBS values for all groups are presented in Table 2. Two-way ANOVA was conducted to examine the interaction between the effect of TAP application and the type of adhesive on MTBS of adhesives to coronal dentin. There was a statistically significant interaction between the effect of dentin treatment and adhesive on MTBS to coronal dentin ( $p = 0.003$ ). It was shown that different types of adhesives significantly affected the MTBS ( $p < 0.001$ ). However, TAP application showed no significant

**Table 1.** Materials used in the study

Material	Category	Product	Composition
Composite resin	Silorane composite resin	Filtek P90 (3M ESPE)	Silorane resin, fine quartz and radiopaque yttrium fluoride
	Methacrylate composite resin	Filtek Z350 (3M ESPE)	BisGMA, TEGDMA, UDMA, silica nanofillers
Bonding system	Self-etch adhesive	Filtek P90 adhesive (3M ESPE)	Primer: Phosphorylated methacrylate, Vitrebond copolymer, bisGMA, HEMA, water, ethanol, silane-treated silica filler, initiator Bondind resin: Hydrophobic dimethacrylate, phosphorylated methacrylates, TEGDMA, silane-treated silica filler, initiators, stabilizers
	Etch-and-rinse adhesive	Adper Single Bond Plus (3M ESPE)	Etchant: 35% phosphoric acid gel Adhesive: Dimethacrylates, HEMA, polyalkenoid acid copolymer, 5 nm silane treated colloidal silica, ethanol, water, photoinitiator

BisGMA, Bisphenol A-glycidyl methacrylate; TEGDMA, Triethyleneglycol-dimethacrylate; UDMA, Urethane dimethacrylate; HEMA, Hydroxyethyl methacrylate.



**Figure 2.** Schematic diagram of the specimen preparation for the microtensile bond strength test. (a) Tooth decoronation below the cemento-enamel junction; (b) Preparation of a flat dentin surface for bonding; (c) Dentin treatment; (d) Adhesive application; (e) Incremental resin composite buildup; (f) Cutting slab from the center of a bonded specimen after 24 h water storage; (g) A single slab containing the resin-dentin interface; (h) Beams for testing from the center slab; (i) attachment of a beam for bond strength evaluation. TAP, tri-antibiotic paste.

**Table 2.** Mean microtensile bond strength values and standard deviations expressed in MPa of different adhesive systems for experimental and control groups ( $n = 30$ )

Adhesive system	Control group	TAP-treated group
Adper Single Bond Plus + Filtek Z350	$37.5 \pm 4.2^a$	$35.8 \pm 4.5^a$
Filtek P90 system	$27.0 \pm 5.6^b$	$21.1 \pm 4.6^c$

TAP, tri-antibiotic paste.

effect on the MTBS ( $p = 0.064$ ). Control specimens in the Adper Single Bond Plus and TAP-treated specimens in the Filtek P90 groups showed the highest and the lowest bond strengths, respectively. Adper Single Bond Plus showed significantly higher mean MTBS than Filtek P90 adhesive in both experimental and control groups regardless of the dentin treatment ( $p < 0.05$ ).

Failure modes are shown in Table 3. For Adper Single Bond Plus, most of the failures in both control and experimental groups were cohesive. There was no significant differences in the mode of failure between experimental and control groups ( $p > 0.05$ ). For Filtek P90 adhesive, there was a

significant difference between experimental and control groups ( $p = 0.038$ ). The control and the experimental groups exhibited a majority of mixed and adhesive failures, respectively.

## Discussion

The coronal seal plays a significant role on the final success of root-filled teeth. Lack of proper adhesion between restorative materials and access cavity in endodontically treated teeth may result in microleakage and compromise prognosis.<sup>19</sup> Inevitable contact of

**Table 3.** Mode of failure of adhesive systems bonded to saline- (control) and TAP-treated dentin ( $n = 30$ )

Failure mode	Adper Single Bond Plus		Filtek P90 adhesive	
	Control group	TAP-treated group	Control group	TAP-treated group
Adhesive	3	3	6	14
Cohesive	17	16	12	9
Mixed	10	11	12	7

Adhesive, adhesive (interface) failure between adhesive and dentin; Cohesive, cohesive failure in composite resin or dentin; Mixed, mixed failure of adhesive and cohesive failures.

TAP, tri-antibiotic paste.

endodontic materials during chemomechanical preparation of root canals may modify the dentin available for coronal restorations and jeopardize bonding procedures.<sup>4,20</sup> Following increased use of TAP due to its sterilization efficacy in endodontic treatments, we assessed the effect of TAP application on the bond strength of two types of etch-and-rinse and self-adhesive dental adhesives to coronal dentin.<sup>2</sup> Results showed no significant differences between control and experimental groups and, therefore, our null hypothesis was rejected.

TAP contains ciprofloxacin, metronidazole, and minocycline. Ciprofloxacin is a synthetic fluoroquinolone with rapid bactericidal action against gram-negative bacteria but with limited activity against gram-positive bacteria.<sup>21</sup> For that reason, ciprofloxacin is often combined with metronidazole for treatment of mixed infections like root canal infections. Metronidazole is a nitroimidazole compound that exhibits broad spectrum of activity against protozoa and anaerobic bacteria.<sup>21</sup> Minocycline exhibits broad spectrum of activity against gram positive and gram negative microorganisms. The optimal antimicrobial effect of TAP seems to occur between seven and 10 days, which was the application time chosen in our study.<sup>2</sup>

The demineralization effect of TAP has been confirmed by significant reduction in phosphate/amide ratio in TAP-treated dentin.<sup>5</sup> The low pH (2.8) of this medicament and the ability of minocycline to chelate calcium and demineralize dental hard tissues may explain its significant demineralization effect.<sup>22</sup> In the present study, although results are not significantly different, the MTBS of both etch-and-rinse and self-etch adhesives tended to be lower in the TAP-treated groups compared to the control groups. This tendency was more obvious in the self-etch group (22% reduction in mean MTBS) compared to the etch-and-rinse group (5.4% reduction in mean MTBS). Excessive demineralization of dentin prior to self-etch adhesive application may prevent optimal adhesive impregnation affecting MTBS as shown by Van Landuyt *et al.*<sup>23</sup>

In the present study, the etch-and-rinse adhesive system Adper Single Bond Plus showed significantly higher MTBS

than that of the self-etch adhesive Filtek P90, irrespective of dentin treatment. Filtek P90 adhesive is a self-etch adhesive (pH = 2), which gently demineralizes the tooth structure resulting in a very thin micro-retentive pattern.<sup>24</sup> This limits the ability of the bonding resin to penetrate the primed surface and might explain the inferior bond strengths of Filtek P90 adhesive compared to the etch-and-rinse adhesive in this study. Potential increased demineralization caused by TAP was not sufficient to increase the MTBS of Filtek P90 adhesive.

Mean MTBS in the present study ranged from 21.1 to 37.5 MPa. It is often not possible to differentiate between the strength of the adhesive and that of the composite resin or dentin when bond strengths exceed values of 18 - 20 MPa because of the increased likelihood of cohesive failure within the dentin.<sup>25</sup> For the self-etch adhesive Filtek P90, the majority of the failures in the experimental group was adhesive with significant differences being noticed between experimental and control groups. Higher number of adhesive failures occurred in the TAP-treated group. The potential lack of proper dentin infiltration may explain the finding.<sup>26</sup> Regarding Adper Single Bond Plus, a higher number of failures were cohesive suggesting that the bond strength between composite resin and dentin was greater than the cohesive strength of the composite resin or dentin itself.

Limitations of our study include the use of only one concentration and application time of TAP, the use of only ethanol/water-based adhesive systems, and the lack of aging of specimens. Furthermore the different characteristics of adhesive resins in the study (methacrylate-based versus silorane-based) could have affected the outcomes of the study. Also, the next session following TAP application may be the cleaning of the root canals with NaOCl and EDTA first. Considering the fact that no studies have investigated the possible interaction between NaOCl and EDTA with TAP, we used normal saline after 10 days to rinse the TAP. Regarding the substrate for bonding, we opted for using coronal dentin considering the similar results between radicular and coronal dentin

in bonding studies.<sup>2,27,28</sup> However, due to the fact that TAP might be directly applied in the canal below the orifice level, it seems rational to also consider radicular dentin in examining the possible effect of TAP on bond strength of adhesive resins when it comes to further studies.

## Conclusions

The etch-and-rinse adhesive Adper Single Bond Plus presented significantly higher mean bond strengths than the self-etch adhesive Filtek P90, irrespective of the group. The superior bond performance of Adper Single Bond was confirmed by a fewer number of adhesive failures when compared to Filtek P90 adhesive. The influence of TAP in bond strength is insignificant.

Conflict of Interest: No potential conflict of interest relevant to this article was reported.

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